Engineered Nanomaterials: Linking Physicochemical Properties with Biology

by

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Outline

- Over view of the safety concern of nanomaterials
- Challenge of knowing toxicity of nanomaterials
- BOD/ROS, nano exposure and adverse health effect
- Developing a screening test to predict toxicity of nanomaterials
- Linking Physicochemical Properties with Biology

“We are in danger of repeating old, potentially costly, mistakes.”

Complexity of Nanomaterials

Uncertainty of Nanoparticle-Biomolecule Interaction
A. Basic Categories
e.g. carbon base materials, metal oxides, elemental metals, Quantum dots, complex compounds, organic polymers, etc.

B. Physical Characteristics
e.g. morphology, diameter, length, aspect ratio, crystallinity, etc.

C. Surface Modification
e.g. surface functionalization, coating, etc.

D. Formation of Secondary Structure by Agglomeration
e.g. morphology, surface charge, hydrophobicity, surface reactivity

Increasing number of possibilities for different ENM's
Challenge of Knowing Toxicity of Nanomaterials

Physicochemical properties of nanomaterials &
The interactions between these properties

Interaction with biomolecules & cells
Distribution
Degradation / Accumulation
Toxicity / Adverse Health Effects

Huge Uncertainty
Challenge of Knowing Toxicity of Nanomaterials

- The link between PCs and toxicity remains poorly understood
- Robust screening approaches are still lacking
- What could be a key metric for screening test?
- How to quantify the key metric and estimate the potential toxicity?
The Possible Mechanisms of Nanotoxicity

- Oxidative stress
- Catalytic Metal in ENMs - catalyze reactive oxygen species generation toxic metal itself
Examples of Particle-mediated Oxygen Radical production

- TiO$_2$
- UV
- H$_2$O
- O$_2$
- O$_2^-$
- e$^-$
- h$^+$
- Electron-donor/acceptor active groups
- Semiconductor properties
- Electron hole pairs
- Dissolution
- Release of ions
- Redox cycling and catalytic chemistry
- OH$^-$
- H$_2$O$_2$
- Fe$^{++}$
- Q
- Q$^-$
- Redox cycling organics
- Ambient UFP Metal NP Carbon NT

Nel et al. Science. 2006
The Possible Mechanisms of Nanotoxicity

- Oxidative stress
- Catalytic Metal in ENMs - catalyze reactive oxygen species generation toxic metal itself
- Membrane disruption – relate to oxidative stress & adsorption
- Essential nutrient or functional biomolecule depletion
- Structure alteration of functional biomolecules
- Others; immune toxicity
Criteria of a Toxicity Screening Test

- Must be sensitive to a large number of physicochemical properties of diverse classes of ENMs that may elicit adverse effects in biological systems.
- Must be highly predictive of potential toxicity of multiple mechanisms.
- Must be relatively simple, sensitive, specific, robust, precise, low cost, exhibit low susceptibility to interferences and possess high throughput capability.
- Must be easily standardized to a highly recognizable endpoint.
“Toxicity Screening tests for new nanomaterials products are urgently needed. Whilst recognizing that oxidative stress potential may not be predictive of all possible adverse outcomes, tests based upon oxidative potential maybe an invaluable tool for initial screening and classification of the relative biohazard of such materials.”
# The human study on association of particulate matter and diseases

<table>
<thead>
<tr>
<th>Title</th>
<th>Journal</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ambient Particulate Pollutants in the Ultrafine Range Promote Early <strong>Atherosclerosis</strong> and Systemic <strong>Oxidative Stress</strong></td>
<td>Circ. Res</td>
<td>Araujo et al. 2008</td>
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<td>Effects of air pollution on the incidence of <strong>myocardial infarction</strong></td>
<td>Heart</td>
<td>Bhaskaran et al. 2009</td>
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<td>Long-Term Exposure to Air Pollution and Incidence of <strong>Cardiovascular Events in Women</strong></td>
<td>N. Engl. J. Med</td>
<td>Miller et al. 2007</td>
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<td><strong>Cardiovascular Mortality</strong> and Long-Term Exposure to Particulate Air Pollution</td>
<td>Circulation</td>
<td>Pope et al. 2004</td>
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<td>Long-term exposure to traffic-related air pollution and <strong>mortality</strong> in Shizuoka, Japan</td>
<td>Occup. Environ. Med</td>
<td>Yorifuji et al. 2010</td>
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Oxidative Damage or ROS Generation Could Be Used as a Metric for Nanotoxicity Screening

2. How to quantify oxidative stress or ROS generation?
## Assay Methods to Determine Reactive Oxygen Spices Generation

<table>
<thead>
<tr>
<th>Assay</th>
<th>Target ROS</th>
<th>Advantages</th>
<th>Disadvantages</th>
<th>used in nano study</th>
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<tr>
<td>DCDHF</td>
<td>ROS</td>
<td>Can be applied intra- and extra-cellularly</td>
<td>Autocatalytic degradation, no information about ROS</td>
<td>√</td>
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<tr>
<td>ESR/EPR</td>
<td>Free radicals</td>
<td>Quantitative, structural information</td>
<td>in virto only/proficiency required</td>
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### Antioxidants Inhibition

<table>
<thead>
<tr>
<th>Assay</th>
<th>Target ROS</th>
<th>Advantages</th>
<th>Disadvantages</th>
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<tbody>
<tr>
<td>FRAS</td>
<td>any type of ROS</td>
<td>Can be applied extra-cellularly</td>
<td>Little information about radical species</td>
</tr>
<tr>
<td>DTT consumption</td>
<td>any type of ROS</td>
<td>Can be applied extra-cellularly</td>
<td></td>
</tr>
<tr>
<td>Vitamin C yellowing</td>
<td>any type of ROS</td>
<td>Can be applied extra-cellularly</td>
<td></td>
</tr>
<tr>
<td>Chemiluminescence (salicylate catalyst)</td>
<td>ROS, •OH and ONOO−</td>
<td>Quantitative</td>
<td>Limited to •OH and ONOO−</td>
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</table>
DCFH vs. FRAS: Comparison
DCFH Method

- NaOH
- ENMs, H$_2$O$_2$, etc.
- Fluorescent compound
Antioxidants in the serum sample

Fe^{+++} \rightarrow \text{blue color} \rightarrow \text{Decrease absorbance} \rightarrow \text{Oxidant Damage}

Nanoparticles

FRAS - Ferric Reducing Ability of Serum Assay

2,4,6-Tripyridyl-1,3,5-Triazine (TPTZ)
Standard Procedures of the FRAS Assay to Measure Oxidative Damage Induced by ENMs

1. Testing media – blood serum
2. Expose blood serum to selected ENMs (10mg mL\(^{-1}\), 37\(^\circ\)C, and 90 min)
3. Remove NPs by two step centrifugations (14,500 g for 15 min)
4. Measure antioxidant capacity of ENMs exposed serum by FRAS
DCFH Assay Results

Positives: 10/28
Negatives: 14/28
Inconclusive: 4/28

Error Bars: 95% CI

Blank
FRAS Assay Results

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<th>Category</th>
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<td>Negatives</td>
<td>4/28</td>
<td>14%</td>
</tr>
<tr>
<td>Inconclusive</td>
<td>3/28</td>
<td>11%</td>
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![Graph showing assay results with error bars for 95% CI.](image)
### DCFH vs. FRAS: Comparison

- FRAS gives positive result in every case DCFH does
- DCFH gives negative result in every case FRAS does
- FRAS never gives a negative result when DCFH gives a positive
- FRAS detects several positive results that DCFH fails to detect

⇒ **FRAS has greater sensitivity across the board**

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<th>FRAS Positives (21/28)</th>
<th>FRAS Negatives (7/28)</th>
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<td>DCFH</td>
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<td></td>
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<td>0</td>
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<tr>
<td></td>
<td>Negatives (18/28)</td>
<td>11</td>
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<tr>
<td></td>
<td></td>
<td>7</td>
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DCFH: Dose-Response

ROS ($H_2O_2$ Eq.)

ENMs
FRAS: Dose-Response

![Graph showing dose-response relationship with linear regression line and R² value]

\[ y = 186.29x \]

\[ R^2 = 0.9964 \]
Linking Physicochemical Properties with Biology

- Physicochemical parameters
- Biological oxidative damage
- Cytotoxicity or adverse health effects
Standard Methods to Measure Physiochemical Properties of ENMs

- **Surface area**
  - N2 sorption analysis (Quantachrome Autosorb-3B, 11-point BET)

- **Transition metals in bulk and water extract**
  - Instrumental Neutron Activation Analysis (INAA) and ICP-MS

- **Surface charge and mobility** - Zeta PALS

- **Crystallinity** - X-Ray diffraction

- **Morphology** - TEM & FE-SEM

- **Organic Carbon** – Modified NIOSH 5040

- **PAHs** - EPA method 3546 & GC-MS 8270

  *PAH*-Polycyclic aromatic hydrocarbons
BOD Variations in MWCNTs
BOD Variations in MWCNTs

Excluded two MWCNTS having high surface area
Metal Distribution in MWCNTs
<table>
<thead>
<tr>
<th>Material</th>
<th>BOD (TEUs, µmol/L)</th>
<th>SSA (m²/g)</th>
<th>Fe</th>
<th>Ni</th>
<th>Co</th>
<th>Mo</th>
<th>Mn</th>
<th>La</th>
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<td>445.5</td>
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<td>164</td>
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<td>98</td>
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<td>135.9</td>
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<td>MWCNT_D₃</td>
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<td>99.7</td>
<td>496</td>
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<td>120</td>
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<td>28.9</td>
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<td>Nano-forest I (assay 1)</td>
<td>432</td>
<td>329.7</td>
<td>712</td>
<td>&lt;9.6</td>
<td>&lt;9.6</td>
<td>173</td>
<td>&lt;9.6</td>
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<td>&lt;8.8</td>
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<td>&lt;0.3</td>
<td>8.2</td>
<td>&lt;0.3</td>
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</tbody>
</table>

**Correlation to BOD**

|                  | -0.1 | -0.3 | **0.9** | 0.2 | 0.4 | -0.3 | -0.1 | 0.4 |

**Correlation to sBOD**

|                  | -0.02 | -0.2 | **0.8** | -0.02 | 0.2 | -0.3 | -0.2 | 0.3 |

Fe-iron  
Cr-Chromium  
Co-Cobalt  
Mo-Molybdenum  
Mn-Manganese
sBOD represent BOD induced by one unit surface was calculated as degree of BOD (μmol of trolox equivalent units) generated by one unit surface area (m²) of MWCNT in 1 ml exposed serum.
By Chemical vapor deposition method

Specific Surface Area (m² g⁻¹)
Surface reactivity of CNTs
Surface reactivity of CNTs
Path Forward

Linking Physicochemical Properties with Biology

Physiochemical Characterization

FRAS

Gene Expression (Prokaryotic Cells)

Cellular Toxicity Testing (Eukaryotic Cells)
Acknowledgments

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THANK YOU